

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the Application of:

R. HERRMANN ET AL.

CASE NO.: BB1367 US CNT

APPLICATION NO.: UNKNOWN

GROUP ART UNIT: UNKNOWN

FILED: HERewith

EXAMINER: UNKNOWN

FOR: SCORPION TOXINS

**PRELIMINARY AMENDMENT**

Commissioner of Patents and Trademarks  
Washington, DC 20231

Sir:

Prior to examination, please amend the captioned application as follows and consider the following remarks.

**IN THE SPECIFICATION:**

**Please replace the following paragraphs:**

**Paragraph beginning at page 1, line 3:**

This application is a continuation application of U.S. Application No. 09/599,416, filed June 22, 2000, which claims the benefit of U.S. Provisional Application No. 60/140,227, filed June 22, 1999, whose contents are hereby incorporated by reference.

**Paragraph beginning at page 3, line 26:**

In a third embodiment, this invention concerns an isolated polynucleotide comprising a nucleotide sequence of at least 30 (preferably at least 40, most preferably at least 60) contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, and 19 and the complement of such nucleotide sequences.

**Paragraph beginning at page 4, line 11:**

In an eighth embodiment, the invention concerns a method of obtaining a nucleic acid fragment encoding a substantial portion of a scorpion K-channel agonist polypeptide, comprising the steps of: synthesizing an oligonucleotide primer comprising a nucleotide sequence of at least 30 (preferably at least 40, most preferably at least 60) contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, and 19, and the complement of such nucleotide sequences; and amplifying a nucleic acid fragment (preferably a cDNA inserted in a cloning vector) using the oligonucleotide primer. The amplified nucleic acid fragment preferably will encode a substantial portion of a scorpion K-channel agonist amino acid sequence.

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**Paragraph beginning at page 8, line 22:**

In the context of this disclosure, a number of terms shall be utilized. The terms "polynucleotide", "polynucleotide sequence", "nucleic acid sequence", and "nucleic acid fragment"/"isolated nucleic acid fragment" are used interchangeably herein. These terms encompass nucleotide sequences and the like. A polynucleotide may be a polymer of RNA or DNA that is single- or double-stranded, that optionally contains synthetic, non-natural or altered nucleotide bases. A polynucleotide in the form of a polymer of DNA may be comprised of one or more segments of cDNA, genomic DNA, synthetic DNA, or mixtures thereof. An isolated polynucleotide of the present invention may include at least 30, preferably at least 40, most preferably at least 60 contiguous nucleotides derived from SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, and 19, or the complement of such sequences.

**Paragraph beginning at Page 16, line 1:**

In addition, two short segments of the instant nucleic acid fragments may be used in polymerase chain reaction protocols to amplify longer nucleic acid fragments encoding homologous genes from DNA or RNA. The polymerase chain reaction may also be performed on a library of cloned nucleic acid fragments wherein the sequence of one primer is derived from the instant nucleic acid fragments, and the sequence of the other primer takes advantage of the presence of the polyadenylic acid tracts to the 3' end of the mRNA precursor encoding arthropod genes. Alternatively, the second primer sequence may be based upon sequences derived from the cloning vector. For example, the skilled artisan can follow the RACE protocol (Frohman et al. (1988) *Proc. Natl. Acad. Sci. USA* 85:8998-9002) to generate cDNAs by using PCR to amplify copies of the region between a single point in the transcript and the 3' or 5' end. Primers oriented in the 3' and 5' directions can be designed from the instant sequences. Using commercially available 3' RACE or 5' RACE systems (BRL), specific 3' or 5' cDNA fragments can be isolated (Ohara et al. (1989) *Proc. Natl. Acad. Sci. USA* 86:5673-5677; Loh et al. (1989) *Science* 243:217-220). Products generated by the 3' and 5' RACE procedures can be combined to generate full-length cDNAs (Frohman and Martin (1989) *Techniques* 1:165). Consequently, a polynucleotide comprising a nucleotide sequence of at least 30 (preferably at least 40, most preferably at least 60) contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, and 19 and the complement of such nucleotide sequences may be used in such methods to obtain a nucleic acid fragment encoding a substantial portion of an amino acid sequence of a polypeptide.

**Paragraph beginning at Page 16, line 23:**

The present invention relates to a method of obtaining a nucleic acid fragment encoding a substantial portion of a scorpion K-channel agonist polypeptide, preferably a substantial portion of an arthropod potassium channel blocking toxin 15-1, Bmtx toxin, neurotoxin P2, leiurotoxin I, leiuropeptide I, leiuropeptide III, kaliotoxin 1 precursor or

cobatoxin 1 polypeptide, comprising the steps of: synthesizing an oligonucleotide primer comprising a nucleotide sequence of at least 30 (preferably at least 40, most preferably at least 60) contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, and 19, and the complement of such nucleotide sequences; and amplifying a nucleic acid fragment (preferably a cDNA inserted in a cloning vector) using the oligonucleotide primer. The amplified nucleic acid fragment preferably will encode a substantial portion of a potassium channel blocking toxin 15-1, a Bmtx toxin, a neurotoxin P2, a leiurotoxin I, a leiuropeptide I, a leiuropeptide III, a kaliotoxin 1 precursor or a cobatoxin 1.

#### IN THE CLAIMS:

**Please cancel claims 1-17.**

**Please add the following claims:**

18. "added" An isolated polynucleotide comprising:
  - (a) a nucleotide sequence encoding a polypeptide having cobatoxin activity, wherein the amino acid sequence of the polypeptide and the amino acid sequence of SEQ ID NO:2 have at least 80% sequence identity based on the Clustal alignment method, or
  - (b) the complement of the nucleotide sequence, wherein the complement and the nucleotide sequence contain the same number of nucleotides and are 100% complementary.
19. "added" The polynucleotide of Claim 18 wherein the amino acid sequence of the polypeptide and the amino acid sequence of SEQ ID NO:2 have at least 85% sequence identity based on the Clustal alignment method.
20. "added" The polynucleotide of Claim 18 wherein the amino acid sequence of the polypeptide and the amino acid sequence of SEQ ID NO:2 have at least 90% sequence identity based on the Clustal alignment method.
21. "added" The polynucleotide of Claim 18 wherein the amino acid sequence of the polypeptide and the amino acid sequence of SEQ ID NO:2 have at least 95% sequence identity based on the Clustal alignment method.
22. "added" The polynucleotide of Claim 18 wherein the polypeptide comprises the amino acid sequence of SEQ ID NO:2.
23. "added" The polynucleotide of claim 18 wherein the nucleotide sequence comprises the nucleotide sequence of SEQ ID NO:1.
24. "added" A vector comprising the polynucleotide of Claim 18.
25. "added" A recombinant DNA construct comprising the polynucleotide of Claim 18 operably linked to a regulatory sequence.
26. "added" A method for transforming a cell comprising transforming a cell with the polynucleotide of Claim 18.
27. "added" A cell comprising the recombinant DNA construct of Claim 25.

28. "added" A method for producing a plant comprising transforming a plant cell with the polynucleotide of Claim 18 and regenerating a plant from the transformed plant cell.

29. "added" A plant comprising the recombinant DNA construct of Claim 25.

30. "added" A method for isolating a polypeptide encoded by the polynucleotide of Claim 18 comprising isolating the polypeptide from a cell containing a recombinant DNA construct comprising the polynucleotide operably linked to a regulatory sequence.

31. "added" An isolated polynucleotide comprising:

(a) a nucleotide sequence encoding a polypeptide having cobatoxin activity, wherein the amino acid sequence of the polypeptide comprises amino acids 22-58 of the amino acid sequence of SEQ ID NO:2, or

(b) the complement of the nucleotide sequence, wherein the complement and the nucleotide sequence contain the same number of nucleotides and are 100% complementary.

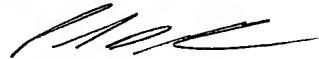
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**REMARKS**

The specification has been amended to include a claim to the benefit of the parent application. In addition, the specification has been amended to correct typographical errors. Furthermore, claims 1-17 have been canceled and claims 18-31 added. No new matter is added by these amendments.

Entry of the amendments and favorable consideration of the claims are respectfully requested.

Respectfully submitted,



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Dated: 1/11/02

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**VERSION WITH MARKINGS TO SHOW CHANGES MADE**

In showing the changes, deleted material is shown as brackets, and inserted material is shown underlined.

**IN THE SPECIFICATION:**

**Paragraph beginning at page 1, line 3:**

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**Paragraph beginning at page 3, line 26:**

In a third embodiment, this invention concerns an isolated polynucleotide comprising a nucleotide sequence of at least [one of 60] 30 (preferably at least [one of] 40, most preferably at least [one of 30] 60) contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, and 19 and the complement of such nucleotide sequences.

**Paragraph beginning at page 4, line 11:**

In an eighth embodiment, the invention concerns a method of obtaining a nucleic acid fragment encoding a substantial portion of a scorpion K-channel agonist polypeptide, comprising the steps of: synthesizing an oligonucleotide primer comprising a nucleotide sequence of at least [one of 60] 30 (preferably at least [one of] 40, most preferably at least [one of 30] 60) contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, and 19, and the complement of such nucleotide sequences; and amplifying a nucleic acid fragment (preferably a cDNA inserted in a cloning vector) using the oligonucleotide primer. The amplified nucleic acid fragment preferably will encode a substantial portion of a scorpion K-channel agonist amino acid sequence.

**Paragraph beginning at page 8, line 22:**

In the context of this disclosure, a number of terms shall be utilized. The terms "polynucleotide", "polynucleotide sequence", "nucleic acid sequence", and "nucleic acid fragment"/"isolated nucleic acid fragment" are used interchangeably herein. These terms encompass nucleotide sequences and the like. A polynucleotide may be a polymer of RNA or DNA that is single- or double-stranded, that optionally contains synthetic, non-natural or altered nucleotide bases. A polynucleotide in the form of a polymer of DNA may be comprised of one or more segments of cDNA, genomic DNA, synthetic DNA, or mixtures thereof. An isolated polynucleotide of the present invention may include at least [one of 60] 30, preferably at least [one of] 40, most preferably at least [one of 30] 60 contiguous nucleotides derived from SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, and 19, or the complement of such sequences.

**Paragraph beginning at Page 16, line 1:**

In addition, two short segments of the instant nucleic acid fragments may be used in polymerase chain reaction protocols to amplify longer nucleic acid fragments encoding homologous genes from DNA or RNA. The polymerase chain reaction may also be performed on a library of cloned nucleic acid fragments wherein the sequence of one primer is derived from the instant nucleic acid fragments, and the sequence of the other primer takes advantage of the presence of the polyadenylic acid tracts to the 3' end of the mRNA precursor encoding arthropod genes. Alternatively, the second primer sequence may be based upon sequences derived from the cloning vector. For example, the skilled artisan can follow the RACE protocol (Frohman et al. (1988) *Proc. Natl. Acad. Sci. USA* 85:8998-9002) to generate cDNAs by using PCR to amplify copies of the region between a single point in the transcript and the 3' or 5' end. Primers oriented in the 3' and 5' directions can be designed from the instant sequences. Using commercially available 3' RACE or 5' RACE systems (BRL), specific 3' or 5' cDNA fragments can be isolated (Ohara et al. (1989) *Proc. Natl. Acad. Sci. USA* 86:5673-5677; Loh et al. (1989) *Science* 243:217-220). Products generated by the 3' and 5' RACE procedures can be combined to generate full-length cDNAs (Frohman and Martin (1989) *Techniques* 1:165). Consequently, a polynucleotide comprising a nucleotide sequence of at least [one of 60] 30 (preferably at least [one of] 40, most preferably at least [one of 30] 60) contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, and 19 and the complement of such nucleotide sequences may be used in such methods to obtain a nucleic acid fragment encoding a substantial portion of an amino acid sequence of a polypeptide.

**Paragraph beginning at Page 16, line 23:**

The present invention relates to a method of obtaining a nucleic acid fragment encoding a substantial portion of a scorpion K-channel agonist polypeptide, preferably a substantial portion of an arthropod potassium channel blocking toxin 15-1, Bmtx toxin, neurotoxin P2, leiurotoxin I, leiuropeptide I, leiuropeptide III, kaliotoxin 1 precursor or cobatoxin 1 polypeptide, comprising the steps of: synthesizing an oligonucleotide primer comprising a nucleotide sequence of at least [one of 60] 30 (preferably at least [one of] 40, most preferably at least [one of 30] 60) contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, and 19, and the complement of such nucleotide sequences; and amplifying a nucleic acid fragment (preferably a cDNA inserted in a cloning vector) using the oligonucleotide primer. The amplified nucleic acid fragment preferably will encode a substantial portion of a potassium channel blocking toxin 15-1, a Bmtx toxin, a neurotoxin P2, a leiurotoxin I, a leiuropeptide I, a leiuropeptide III, a kaliotoxin 1 precursor or a cobatoxin 1.

**IN THE CLAIMS:**

**Claims 1-17 canceled.**

**Claims 18-31 added.**

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